Extent of Salmonellae Contamination in Primary Breeder Hatcheries in 1998 as Compared to 1991

N. A. COX*,[1], M. E. BERRANG*, and J. M. MAULDIN†
**USDA, ARS, Russell Research Center, 950 College Station Rd., Athens, GA 30605*
†Extension Poultry Science Dept., University of Georgia, Athens, GA 30602
Phone: (706) 546-3484
FAX: (706) 546-3772
e-mail ncox@saa.ars.usda.gov

Primary Audience: Hatchery Managers, Field Service Personnel, Production Managers, Quality Control Personnel

Summary

Eggshell fragments, paper pads from chick boxes, and fluff samples were obtained from three commercial primary breeder hatcheries and analyzed for the presence and level of salmonellae with identical laboratory methods in 1991 and 1998. Overall, 29 of 180 samples (16.1%) from the three hatcheries in 1998 were contaminated with salmonellae, whereas in 1991, 11.1% of the overall samples were found to be salmonellae positive. Salmonellae were detected in 1.7% of eggshell fragments, 1.7% of fluff samples, and 48% of the paper pad samples in 1998, whereas 15.2, 4.5, and 12% of these type samples, respectively, were salmonellae positive in 1991. Although the percentage of positive samples was slightly higher in 1998 than 1991, from an enumeration standpoint, the salmonellae contamination in primary breeder hatcheries seems to have improved in the past 7 yr. In 1998, less than 4% of the positive samples had high levels of salmonellae, whereas in 1991 36% of the positive samples had high numbers of salmonellae. Primary broiler breeder and broiler hatcheries present critical control points in the prevention of salmonellae contamination during commercial poultry production. The cycle of salmonellae contamination will not likely be broken until contamination at these critical points is dramatically reduced or eliminated.

Key words: Broiler breeder, chickpad, eggshell, fluff, hatchery, Salmonella
2001 J. Appl. Poult. Res. 10:202–205

Description of Problem

Freshly laid, fertile eggs are wet, warm, and susceptible to rapid penetration by bacteria (including salmonellae). Contaminated eggs have the potential for spreading salmonellae in the hatchery [1] and subsequently throughout the integrated poultry industry. Unfortunately, commercial hatcheries have been shown to be highly contaminated with salmonellae [2, 3], and these bacteria are able to persist for long periods of time in these hatcheries [4]. To minimize the salmonellae contamination of broiler chickens during production, hatchery contamination (both

[1] To whom correspondence should be addressed.

TABLE 1. Salmonellae contamination in three primary breeder hatcheries sampled[A] in 1998

FACILITY	REPLICATE	SHELLS	PADS	FLUFF
1	1	0/10	6/10	0/10
	2	0/10	1/10	0/10
2	1	0/10	0/10	0/10
	2	1/10	10/10	1/10
3	1	0/10	6/10	0/10
	2	0/10	6/10	0/10
Overall		1/60 (1.7%)	29/60 (48.3%)	1/60 (1.7%)

[A]Number of salmonellae-positive samples per total samples tested.

breeder and broiler hatcheries) must be constantly addressed. By controlling hatchery contamination, it may be possible to eliminate or dramatically reduce salmonellae and other human enteropathogens in the final product.

In a study conducted in our laboratory in 1991 [3], commercial primary breeder hatcheries from six different integrated poultry companies were sampled for the presence and level of salmonellae. The objective of the present study was to return to the same hatcheries and use the same laboratory methods and personnel to measure salmonellae contamination (incidence and level) found in 1998. In doing so, we would be able to compare data from 1991 to data from 1998. We encountered several problems in achieving our objectives. To begin with, four of the previous six primary breeder hatcheries were no longer in operation. Another would not allow us to sample their hatchery in 1998. One of the previously sampled hatcheries was sampled again and two new ones (not previously sampled) were included in the 1998 study. Although the same hatcheries were not sampled in 1991 and 1998, the same researchers using the same methodologies were employed and allowed for comparison of salmonellae contamination in primary breeder hatcheries.

Materials and Methods

In 1998, eggshell fragments, fluff, and paper pads were obtained from each of three commercial breeder hatcheries on each of two sampling days. Approximately 10 g of eggshell fragments were aseptically removed from the hatching trays and placed in a sterile plastic bag containing 90 mL of buffered peptone (BP) medium [5]. Approximately 5 g of fluff was aseptically removed from the hatching cabinet and placed in a sterile plastic bag containing 50 mL of BP. Each paper pad was cut into pieces approximately 4 inches square, using sterile scissors, and was placed in a sterile plastic bag containing 500 mL of BP. Randomly selected samples regardless of sample type, were semi-quantitatively analyzed by removing 0.01 and 0.001 mL of the BP prior to incubation. Samples were incubated separately and then analyzed for the presence of salmonellae as described below.

After overnight incubation of BP at 37°C, 0.1 mL was aseptically transferred to 9 mL of

TABLE 2. Comparison of prevalence of salmonellae-positive samples from a primary breeder hatchery tested in 1991 and 1998

MATERIAL SAMPLED	SAMPLED 1991		SAMPLED 1998	
Eggshell	22/145[A]	(15.2%)[B]	1/60**	(1.7%)
Paper Pads	15/125	(12.0%)	27/60**	(45.0%)
Fluff	5/110	(4.5%)	1/60	(1.7%)
Overall	42/380	(11.1%)	29/180	(16.1%)

[A]Number of salmonellae-positive samples per number of samples tested.
[B]Percentage of salmonellae-positive samples.
**Significant difference in number of positives compared to 1991 samples ($P < 0.01$ by chi-squared test).

TABLE 3. Comparison of the level of salmonellae in positive samples from a primary breeder hatchery sampled in 1991 and 1998

SALMONELLA PER SAMPLE	1991	1998
$>10^3$	36.4%	3.7%
$>10^2$ but $<10^3$	27.3%	0.0%
$<10^2$	36.4%	96.3%

TT broth base [6] and incubated for 24 h at 42°C. Plates of brilliant green sulfa agar [6] and USDA-modified lysine iron agar [7] were streaked for isolation with a loopful (3-mm loop) of TT broth. After incubation of the plates for 24 to 48 h, two suspect colonies appearing on the plates were selected, biochemically screened, and then serologically confirmed to be *Salmonella*. Identical laboratory methods were used for the 1991 and 1998 samples.

Results and Discussion

The results collected in 1998 appear in Table 1. The eggshell and the fluff samples were almost all negative from all the hatcheries (less than 2% positive). However, approximately half of all the paper pad samples were salmonellae positive in these three hatcheries. The only hatchery that was sampled in 1991 and 1998 was Hatchery 1. In 1991, 18/80 (22.5%) samples tested were positive for salmonellae and in 1998 7/60 (11.1%) were positive. Tables 2 and 3 show a comparison of the data from 1991 to 1998 for prevalence and level of salmonellae contamination, respectively. The eggshell and fluff samples had a lower incidence of salmonellae contamination in 1998 than in 1991, whereas the percentage of positive paper pads was higher. Overall, the percentage of salmonellae-positive samples from 1998 was 5% higher than that found in 1991. However, from the only hatchery sampled in both studies, a decrease in the percentage of salmonellae-positive samples was observed (22.5 vs. 11.1%). In addition, the number of salmonellae-positive samples that had greater than 10^3 *Salmonella* per sample was dramatically reduced over the past 7 yr (Table 3). In 1998, less than 4% of the positive samples had high levels of salmonellae, whereas 36% of them did in 1991.

Because salmonellae organisms can enter the hatchling through an assortment of body openings, such as the mouth, nares, navel, eye, or cloaca [4], and very low numbers are required to colonize young birds [8], the observed reduction in salmonellae is very important. Newly hatched chicks that become colonized early in life will subsequently spread salmonellae to other chicks in the hatchery and to flock mates during growout [9]. When these flocks reach the processing plant, salmonellae often contaminates the outside and the inside of these broilers [10] and may ultimately contaminate the fully processed carcasses from this flock and subsequently processed flocks.

Several large-scale field trials have tracked *Salmonella* serotypes originating from the hatchery to the final processed carcass [11, 12, 13]. Our study has clearly shown that salmonellae are present in significant numbers in primary breeder hatcheries [2, 3, 9] and that breeder flocks are early critical control points for preventing salmonellae entry into the integrated poultry industry. Even though the situation in these commercial hatcheries seems to be improving, efforts must be continued or even intensified if the poultry industry is to succeed in producing salmonellae-free poultry. To produce fully processed poultry that is free from salmonellae, birds will have to be hatched, reared, and transported to the processing plant without salmonellae contamination (internal or external), which is not possible at present.

Conclusions and Applications

1. Even though the percentage of positive samples was slightly higher in 1998 than 1991, the salmonellae contamination in primary breeder hatcheries has improved from an enumeration standpoint over the past 7 yr. In 1998, less than 4% of the positive samples had high levels of salmonellae as compared with 36% in 1991.
2. A decrease in the percentage of salmonellae-positive samples was observed in the only primary breeder hatchery that was sampled in 1991 (22.5%) and 1998 (11.1%).

3. Primary breeder hatcheries continue to be a very important critical control point in the transmission of salmonellae to young chicks.

REFERENCES AND NOTES

1. **Williams J.E., and L.H. Dillard,** 1968. *Salmonella* penetration of fertile and infertile chicken eggs at progressive stages of inoculation. Avian Dis. 12:629–635.

2. **Cox, N.A., J.S. Bailey, J.M. Mauldin, and L.C. Blankenship,** 1990. Research note: Presence and impact of *Salmonella* contamination in commercial broiler hatcheries. Poult. Sci. 69:1606–1609.

3. **Cox, N.A., J.S. Bailey, J.M. Mauldin, L.C. Blankenship, and J.L. Wilson,** 1991. Research note: Extent of salmonellae contamination in breeder hatcheries. Poult. Sci. 70:416–418.

4. **Cox, N.A., J.S. Bailey, and M.E. Berrang,** 1996. Alternative routes for *Salmonella* intestinal tract colonization of chicks. J. Appl. Poult. Res. 5:282–288.

5. **Juven, B.J., N.A. Cox, J.S. Bailey, and J.E. Thomson,** 1984. Minimal media recovery of *Salmonella* from dry feed. J. Food Prot. 47:299–302.

6. **Difco Laboratories,** Division of Becton Dickinson and Co., Sparks, MD 21152.

7. **Oxoid Inc.,** 217 Colonnade Road, Nepean, Ontario K2E 7K3, Canada.

8. **Cox, N.A., J.S. Bailey, L.C. Blankenship, R.J. Meinersmann, N.J. Stern, and F. McHan,** 1990. Research note: Fifty percent colonization dose for *Salmonella typhimurium* administered orally and intracloacally to young broiler chicks. Poult. Sci. 69:1809–1812.

9. **Cox, N.A., J.S. Bailey, M.E. Berrang, and J.M. Mauldin,** 1997. Diminishing incidence and level of salmonellae in commercial broiler hatcheries. J. Appl. Poult. Res. 6:90–93.

10. **Bailey, J.S., N.A. Cox, and L.C. Blankenship,** 1990. Persistence and spread of external *Salmonella* contamination during broiler production. Poult. Sci. 69(Suppl. 1):154.

12. **Bhatia, T.R., and G.D. McNabb,** 1980. Dissemination of *Salmonella* in broiler-chicken operations. Avian Dis. 24:616–624.

13. **Goren, E., W.A. de Jong, P. Doornenbal, N.M. Bolder, R.W. Mulder, and A. Jansen,** 1988. Reduction of *Salmonella* infection of broilers by spray application of intestinal microflora: a longitudinal study. Vet. Q. 10:249–255.

14. **Blankenship L.C., J.S. Bailey, N.A. Cox, N.J. Stern, R. Brewer, and O. Williams,** 1993. Two-step mucosal competitive exclusion flora treatment to diminish salmonellae in commercial broiler chickens. Poult. Sci. 72:1667–1672.

ACKNOWLEDGMENTS

Outstanding technical assistance was received from D. Posey, and clerical assistance was from C. Williams.